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(54) Device and methods for mixing fluid contained in a cartridge

(57) The invention provides exemplary devices, systems and methods for mixing of fluids, such as biological fluids. One exemplary device (10) comprises is particularly adapted for holding at least one cartridge (48) having a chamber (58) containing a biological fluid, with the chamber (58) having a generally planar face (60). The device (10) comprises a rotatable body (12) having a rotational axis, a base (14), a plurality of parallel spaced apart walls (16,18 and 20) and two end pieces (22,24). The walls (16 and 20) each include a plurality of slots (44). Slots (44) in each of walls (16 and 20) oppose a corresponding slot (46) in wall (18). In this way, a cartridge (48) may be removably mounted to body (12) by inserting the cartridge between a pair of opposing slots (44 and 46).

The rotatable body (12) includes at least one mounting element which is adapted to mount the rotatable body (12) such that the face of the chamber (58) is generally perpendicular to the rotational axis.

Although useful in mixing a wide variety of biological fluids, the device will find its greatest use when mixing a sample within a hybridization chamber having a polymer array.

The device may also be useful in facilitating a variety of reactions where mixing is required, including, e.g., extension or amplification reactions using tethered probes as template or primer sequences, screening of receptors against arrays of small molecules, peptides or peptideomimetics, carbohydrates and the like.

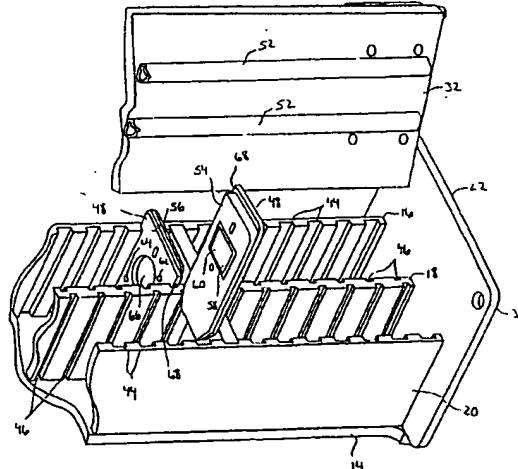


FIG - 5

Description

[0001] This application is a continuation-in-part of U. S. Patent Application Serial No. 09/016,564, filed January 30, 1998, the full disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] This invention relates generally to the field of mixing, and in particular to the mixing of fluids. In one particular aspect, the invention relates to the mixing of biological fluids within a chamber which is disposed within a heated environment.

[0003] Methods for using arrays of polymers to identify receptors with specific affinities for one of the polymers in the array are known. For example, one method uses immobilized antibodies to analyze binding to peptide ligands or vice-versa. Another type of method uses immobilized oligonucleotides to analyze hybridization to a target nucleic acid. For instance, U.S. Patent Application Serial No. 08/624,312, filed March 26, 1996, the complete disclosure of which is herein incorporated by reference, describes apparatus and methods for carrying out repeated hybridizations of a target nucleic acid to an array of nucleic acid probes. Such polymer arrays are described in, e.g., U.S. Patent No. 5,143,854 and published PCT Application Nos. WO90/15070 and WO92/10092, the complete disclosures of which are herein incorporated by reference. These polymer arrays are nucleic acid arrays which include a plurality of different polynucleotides coupled to a substrate in different known locations.

[0004] In one exemplary arrangement, such arrays are packaged within a housing, like those described in, e.g., pending U.S. Patent Application Serial Nos. 08/624,312, previously incorporated by reference, 08/485,452, filed June 7, 1995, and 08/528,173, filed September 19, 1995, and published PCT Application No. WO95/33846. The disclosures of all of these references are herein incorporated by reference. In brief, such a housing typically includes a body having a reaction cavity or hybridization chamber. The array or substrate is mounted over the cavity on the body such that the front side of the array substrate, e.g., the side upon which the polynucleotides are situated, is in fluid communication with the cavity. The cartridge includes inlet and outlet ports to allow various fluids to be introduced into and removed from the hybridization chamber.

[0005] During hybridization, it is often desirable to provide an efficient and effective way to mix the fluids within the chamber. This can be challenging since the chamber is typically in a temperature controlled environment, such as in an oven. Additionally, in at least one embodiment, the interior of the chamber is narrow, and it can be difficult to mix or agitate the fluid when within the chamber.

[0006] Hence, it would be desirable to provide devic-

es, systems and methods to facilitate the mixing of fluids which are held within a hybridization chamber to improve the hybridization process. Such devices, systems and methods should be cost effective, easy to use, and be compatible with the constraints of the hybridization process. In particular, it would be desirable if mixing of the fluid could be facilitated while the chamber is within a temperature controlled environment, such as an oven.

10 SUMMARY OF THE INVENTION

[0007] The invention provides systems, devices and methods for facilitating the mixing of various fluids within a chamber. In one exemplary embodiment, a system is

15 provided which comprises at least one cartridge having a chamber for holding a fluid (which is preferably a biological fluid), with the chamber including a generally planar face. The system further includes a rotatable body having a rotational axis. The rotatable body includes at least one mounting element to removably mount the cartridge to the rotatable body such that the face of the chamber is generally perpendicular to the rotational axis.

20 [0008] Preferably, the mounting element comprises a pair of opposing walls which each include at least one slot. The slots are arranged such that the cartridge is insertable into the slots. Conveniently, the rotatable body may also include a base which connects the pair of walls. Preferably, the rotational axis extends through one of the walls. In this way, the cartridge will be spaced apart from the rotational axis to facilitate mixing of the fluid when the body is rotated.

25 [0009] Preferably, the opposing walls are parallel to each other and the slots are configured such that the face of the chamber is generally perpendicular to the walls when placed into the slots. Preferably, at least one of the slots is keyed such that the cartridge is insertable into the slot in only one orientation. Optionally, each wall may include a plurality of slots which are arranged such that multiple cartridges may be inserted into the slots in a parallel arrangement. It will be appreciated that the rotatable body may include additional numbers of parallel walls which each include slots so that multiple cartridges may be removably mounted to the body.

30 [0010] In one aspect, a lid is also provided and is operably attached to at least one of the walls. The lid is movable between an open and a closed position, with the lid securing the cartridge within the slots when the lid is in the closed position. At least one coupling element is preferably operably attached to the body in alignment with the rotational axis. In this way, the coupling element may couple the rotatable body to a rotation mechanism.

35 [0011] In one particular aspect, the chamber includes a pair of planar spaced apart faces which define an interior having the fluid. The faces are generally rectangular or square in geometry, and the cartridge is mounted to the body such that the faces are generally perpen-

dicular to the rotational axis to facilitate mixing of the fluid within the chamber.

[0012] The body will preferably be rotated about the rotational axis at a rate in the range from about 30 rpm to about 90 rpm, and more preferably from about 50 rpm to about 60 rpm. Further, the rotational body will preferably be placed within a temperature controlled environment during rotation of the body. In one particular aspect, the chamber is heated to a temperature in the range from about 30 °C to about 60 °C, and more preferably from about 40 °C to about 50 °C, while the body is rotating. Such heating may be accomplished, for example, by placing the rotatable body within an oven.

[0013] In a preferred aspect, the device will be fabricated of transparent plexiglass or other suitable lightweight, rigid, machinable material. preferably the device will be a generally amber color. Preferably the device will filter out wavelengths of light in the range of 200 nm to 700 nm.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Fig. 1 is a perspective view of an exemplary device for holding multiple cartridges which each include a hybridization chamber according to the invention.

[0015] Fig. 2 is a top view of the device of Fig. 1.

[0016] Fig. 3 is a right side view of the device of Fig. 1.

[0017] Fig. 4 is a left side view of the device of Fig. 1.

[0018] Fig. 5 is a partial cutaway view of the device of Fig. 1 showing a cartridge being inserted into the device according to the invention.

[0019] Fig. 6 illustrates a method for rotating the device of Fig. 1 after cartridges have been inserted into the device according to the invention.

DETAIL DESCRIPTION OF THE SPECIFIC EMBODIMENTS

[0020] The invention provides exemplary devices, systems and methods to facilitate the mixing of fluids that are held within a chamber, and will preferably be used in connection with biological fluids. Although useful in mixing a wide variety of biological fluids, the invention will find its greatest use when mixing a sample within a hybridization chamber having a polymer array. Although the invention will find particular use in connection with hybridization reactions and, more specifically, nucleic acid hybridizations, it should be appreciated that the invention will be useful in facilitating a variety of reactions where mixing is required, including, e.g., extension or amplification reactions using tethered probes as template or primer sequences, screening of receptors against arrays of small molecules, peptides or peptidomimetics, carbohydrates, and the like.

[0021] The invention will find use in facilitating the mixing of fluids within chambers having a wide variety of configurations and geometries. However, the inven-

tion will be particularly useful with chambers which are relatively narrow in geometry, e.g., defined by two closely spaced apart planar walls. Such chambers are described in co-pending U.S. Application Serial Nos. 5 08/624,312, 08/485,452 and 08/528,173 and PCT Application No. WO95/33846, previously incorporated herein by reference.

[0022] The invention provides for the mixing of the various fluids by rotating the chambers about a rotational axis that is generally perpendicular to the narrow chamber. Rotation in this manner is particularly advantageous when the chamber includes corners, such as in a rectangular or square chamber. When rotating the chamber about the rotational axis, the fluid within the chamber will become agitated as the direction of flow is hindered due to the change in direction of the walls. In this way, mixing of the fluid is facilitated.

[0023] According to the invention, mixing will preferably occur while the chamber is within a temperature controlled environment. Typically, the chamber will be included within an oven so that the chamber may be heated while the chamber is being rotated. For example, one such oven which may be modified to receive a rotating chamber is a General Purpose Incubator, Model 1535, 25 commercially available from VWR Scientific Products, West Chester, PA.

[0024] Referring now to the figures, an exemplary embodiment of a device 10 for holding cartridges having a chamber containing a fluid will be described. The fluid held within the chamber will preferably comprise a biological fluid. The cartridges will preferably be of the type described generally in co-pending U.S. Application Serial Nos. 08/624,312, 08/485,452 and 08/528,173 and PCT Application No. WO95/33846, previously incorporated by reference. Such cartridges are illustrated generally in Fig. 5 and will be described in greater detail hereinafter.

[0025] Device 10 comprises a rotational body 12 having a base 14, a plurality of parallel spaced apart walls 16, 18 and 20, and two end pieces 22 and 24. As best shown in Figs. 2-4, coupling elements 26 and 28 are attached to end pieces 22 and 24, respectively. Extending between coupling elements 26 and 28 (and through wall 18) is a rotational axis about which body 12 is rotated as described in greater detail hereinafter.

[0026] End pieces 22 and 24 each include a set of apertures 30 as best shown in Fig. 1. Apertures 30 are employed to couple a lid 32 to end pieces 22 and 24. As shown in Fig. 1, lid 32 is in an open position. In Figs. 50 2-4, lid 32 is in a closed position. Lid 32 is coupled to end pieces 22 and 24 by rods 34, which in turn are attached to lid 32 by brackets 36. A spring 38 is disposed within each of brackets 36 to bias rods 34 toward their respective aperture 30 so that lid 32 will be secured to end pieces 22 and 24 when in the closed position.

[0027] The elements used to construct body 12 will preferably comprise a generally rigid, heat resistant material that may withstand temperatures that are within

the range of from about 30 °C to about 60 °C, and more preferably from about 40 °C to about 50 °C. Conveniently, the elements used to construct body 12 comprise a plastic material, with a preferable material being sold under the trade name of Plexiglass. Other suitable lightweight, rigid, machinable, heat resistant materials including acrylic, lucite, styrene, polystyrene, and polycarbonate may also be used. Conveniently, the elements may be constructed to be essentially transparent so that visualization into body 12 may be facilitated. In a preferred embodiment, the elements are generally amber in color such that wavelengths of light in the range of 200 nm to 700 nm, and more preferably 250 nm to 600 nm, will be prevented from passing therethrough.

[0028] As best shown in Fig. 3, coupling element 26 includes a cylindrical hole 40 which is aligned with the rotational axis. Hole 40 is adapted to receive a rotatable shaft so that, as the shaft is rotated, body 12 will rotate about the rotational axis. As shown in Fig. 2, coupling element 28 includes a rotatable sleeve 42 which is adapted to rest upon a bracket (not shown) so that body 12 will rotate about the rotational axis when the shaft in hole 40 is rotated. An exemplary motor unit for rotating body 12 comprises a Rotamix Rotisserie with Digital Display, commercially available from Appropriate Technical Resources, Laurel Maryland. Preferably, body 12 is coupled to the motor unit, and the motor unit is then placed into the oven or incubator. The motor unit is then actuated to rotate body 12 within the oven.

[0029] Referring now to Figs. 1 and 2, walls 16 and 20 each include a plurality of slots 44. Slots 44 in each of walls 16 and 20 oppose a corresponding slot 46 in wall 18. In this way, a cartridge 48 (see Fig. 5) may be removably mounted to body 12 by inserting the cartridge between a pair of opposing slots 44 and 46. Conveniently, a pair of cross walls 50 are provided to subdivide body 12 into four sections. The division of body 12 into various sections facilitates organization of the various cartridges.

[0030] Each pair of opposing slots 44 and 46 are configured such that when the cartridge is inserted into the slots, the cartridge will be generally perpendicular to walls 16, 18 and 20. As described hereinafter, slots 46 are narrower than slots 44 so that the cartridges can only be inserted in one particular orientation. Conveniently, lid 32 is provided with a pair of rubber strips 52 which serve to securely hold the cartridge within slots 44 and 46 when lid 32 is in the closed position.

[0031] Referring now to Fig. 5, insertion of cartridges 48 into body 12 will be described in greater detail. The particular construction of cartridge 48 is described in co-pending U.S. Application Serial Nos. 08/624,312, 08/485,452 and 08/528,173 and PCT Application No. WO95/33846, previously incorporated by reference. Briefly, cartridge 48 includes a front 54 and a rear 56. Front 54 includes a cavity 58 which is defined by a generally planar face 60. Positioned across cavity 58 is an array chip (not shown). When the array chip is posi-

tioned over cavity 58, a hybridization chamber is formed. The hybridization chamber is generally rectangular or square in geometry and has a narrow width as defined by the distance between planar face 60 and the array chip. An inlet port 62 and an outlet port 64 are included in rear 56 to allow various fluids to be introduced into and removed from the hybridization chamber. Rear 56 further includes a cavity 66, located adjacent the array, which is adapted for receiving a temperature monitoring and/or controlling device employed in other applications.

[0032] Cartridge 48 includes an extending edge 68 which is sized to be received into slots 46, while the opposite side of cartridge 48 is adapted to be received in slots 44. In this way, cartridge 48 can only be inserted between a pair of slots 44 and 46 in one orientation as shown in Fig. 5. In such an orientation, planar face 60 is generally perpendicular to the rotational axis. In this way, as body 12 is rotated, the fluid within the hybridization chamber will flow against each edge of the hybridization chamber to facilitate mixing of the fluid.

[0033] Referring now to Figs. 5 and 6, operation of device 10 to facilitate mixing of a fluid, such as a biological fluid, will be described. Initially, the hybridization chamber within cartridge 48 is at least partially filled with a liquid. Cartridge 48 is then inserted between a pair of opposing slots 44 and 46, with edge 68 being inserted into slot 46. Lid 32 is then closed, with each of rods 34 being received into its respective aperture 30. Strips 52 will be pressed upon cartridge 48 to securely hold the cartridges within the slots. Device 10 is then coupled to a rotation unit and placed within an oven as previously described. The oven is set to the desired temperature, which will typically be in the range from about 30 °C to about 60 °C, and more preferably from about 40 °C to about 50 °C. While within the oven, the rotation mechanism is actuated to rotate body 12 about the rotational axis as illustrated by the arrow in Fig. 6. Preferably, body 12 will be rotated at a rate in the range from about 30 rpm to about 90 rpm, and more preferably from about 50 rpm to about 60 rpm.

[0034] After mixing and heating is completed, device 10 is removed from the oven and lid 32 is opened so that cartridges 48 may be removed. At this point, cartridges 48 may be processed further as is known in the art.

[0035] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent documents were so individually denoted regardless of whether previously incorporated by reference.

Claims

1. A device for holding at least one cartridge having a chamber containing a fluid, wherein the chamber includes a generally planar face, the device comprising:

a rotatable body having a rotational axis, wherein the rotatable body includes at least one mounting element which is adapted to mount the rotatable body such that the face of the chamber is generally perpendicular to the rotational axis.

2. The device as in claim 1, wherein the mounting element comprises a pair of opposing walls which each include at least one slot, wherein the slots are adapted to receive the cartridge, and further comprising at least one coupling element operably attached to the body in alignment with the rotational axis, wherein the coupling element is adapted to couple the rotatable body to a rotation mechanism.

3. The device as in claim 2, wherein the rotatable body comprises a base connecting the pair of walls, wherein the opposing walls are parallel to each other, and wherein the slots are configured such that the face of the chamber is generally perpendicular to the walls when placed into the slots, and further comprising a lid operably attached to at least one of the walls and which is adapted to secure the cartridge within the slots when the lid is closed.

4. The device as in claim 2, wherein the rotational axis extends through one of the walls, and wherein at least one of the slots is keyed such that the cartridge is insertable into the slot in only one orientation.

5. The device as in claim 2, wherein each wall includes a plurality of slots which are adapted to receive a plurality of cartridges.

6. The device as in claim 5, wherein each wall includes a plurality of slots, and wherein the slots are arranged such that multiple cartridges may be inserted into the slots in a parallel arrangement.

7. The device as in claim 2, wherein the rotatable body includes at least three parallel walls, with the walls each including slots which are adapted to receive multiple cartridges.

8. The device as in claim 2, wherein the chamber includes a pair of planar spaced-apart faces which define an interior having the fluid, wherein the faces are generally rectangular in geometry, and wherein the cartridge is mounted to the body such that the faces are generally perpendicular to the rotational axis to facilitate mixing of the fluid within the chamber.

9. The device as in claim 1, wherein the device is fabricated from a generally amber coloured transparent material which prevents the passage of light having a wavelength in the range of 200 nm to 700 nm.

10. A system to facilitate the hybridization of a fluid, the system comprising a device according to any previous claim.

11. A method for facilitating the hybridization of a fluid, the method comprising:

providing a cartridge having a chamber at least partially filled with a fluid, wherein the chamber includes a generally planar face;

removably coupling the cartridge to a rotatable body having a rotational axis such that the face of the chamber is generally perpendicular to the rotational axis; and

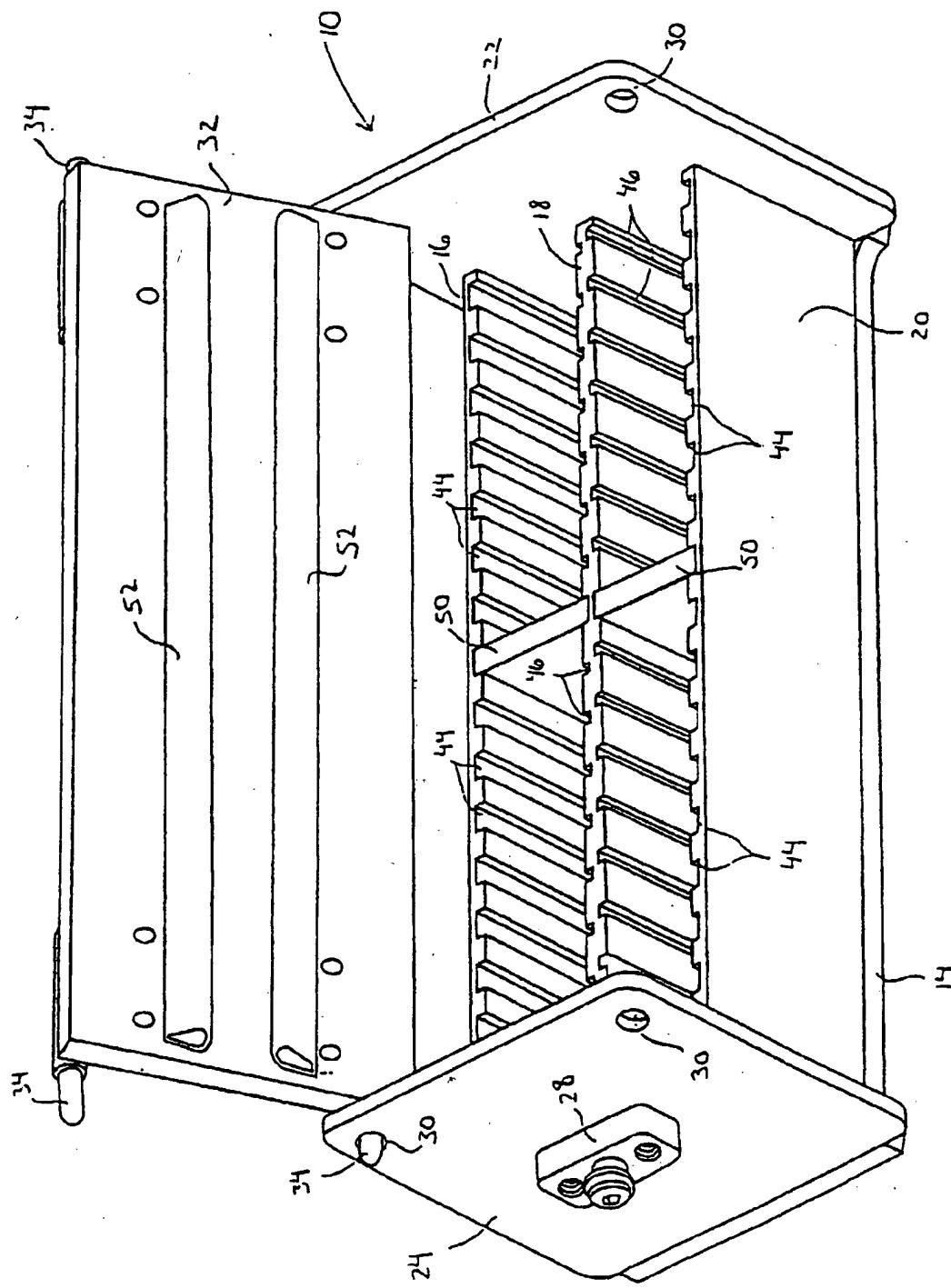
rotating the rotatable body about the rotational axis.

12. The method as in claim 11, further comprising rotating the body about the rotational axis at a rate in the range from about 30rpm to about 90rpm and heating the chamber within an oven to a temperature in the range from about 30 degrees C. to about 60 degrees C while the body is rotating.

13. The method as in claim 11, wherein the coupling step comprises inserting the cartridge into slots formed in a pair of spaced-apart walls, and further comprising securing the cartridge within the slots prior to rotating the body.

14. The method as in claim 11, further comprising inserting multiple cartridges into multiple slots in the pair of walls.

15. The method as in claim 11, wherein the chamber includes a pair of planar spaced-apart faces which define an interior having the fluid, wherein the faces are generally rectangular in geometry, and further comprising coupling the cartridge to the body such that the faces are generally perpendicular to the rotational axis to facilitate mixing of the fluid within the chamber.



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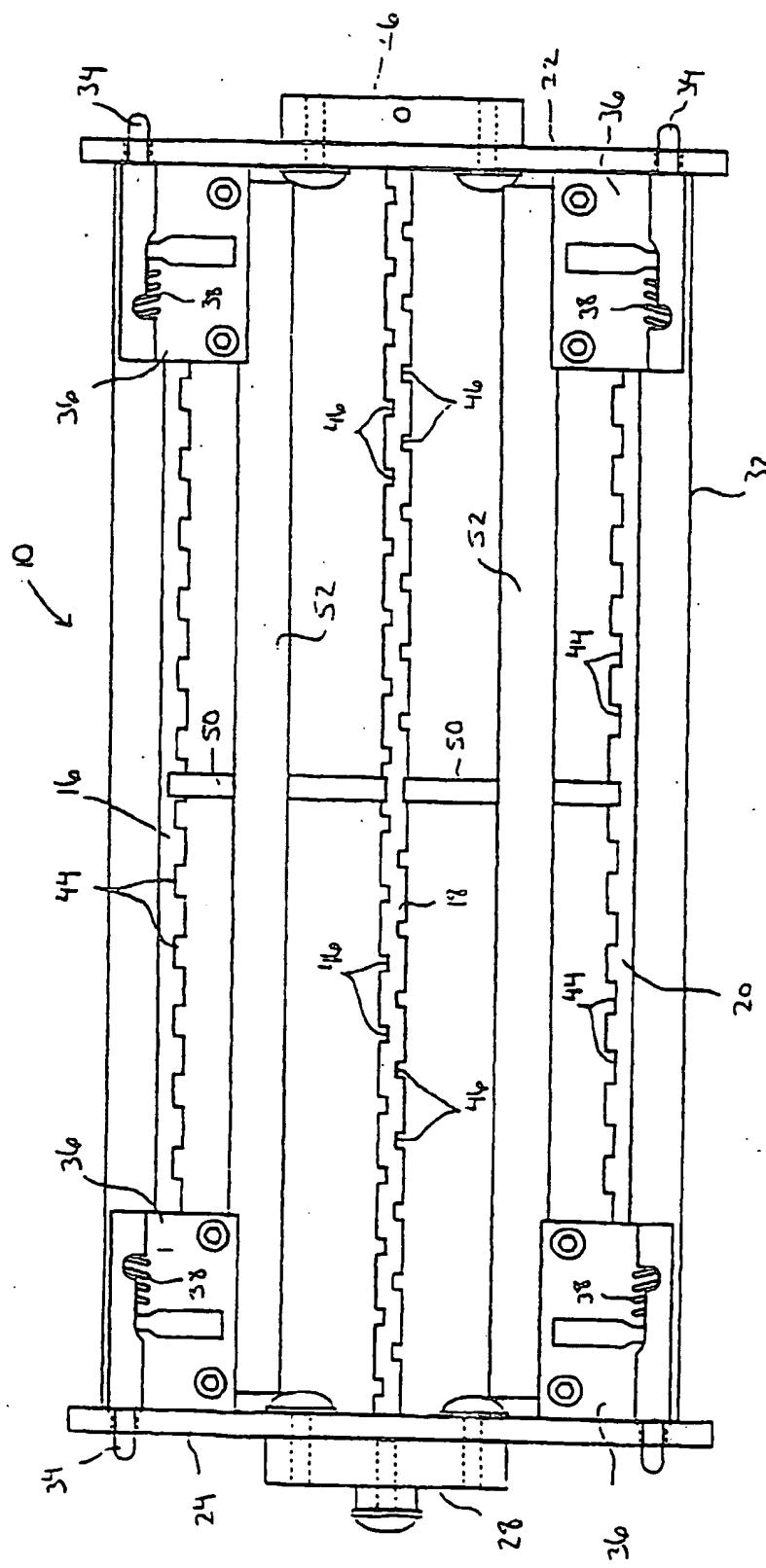


FIG - 2

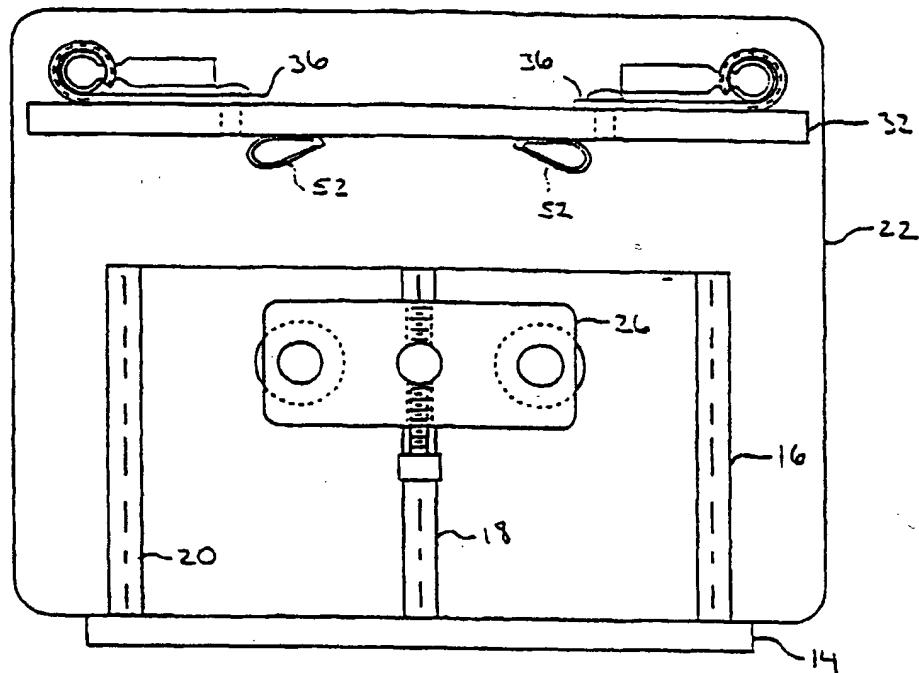


FIG - 3

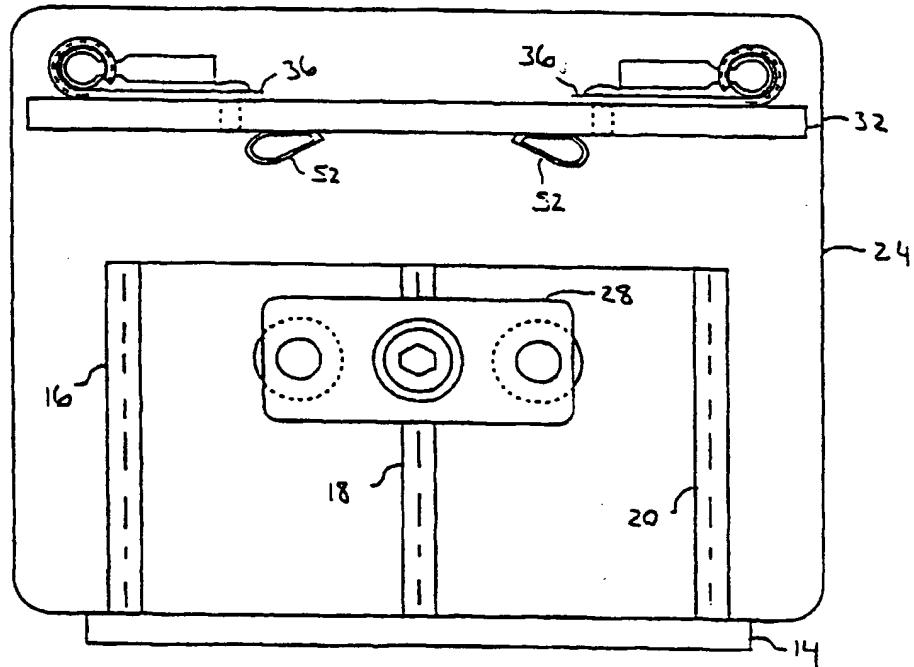
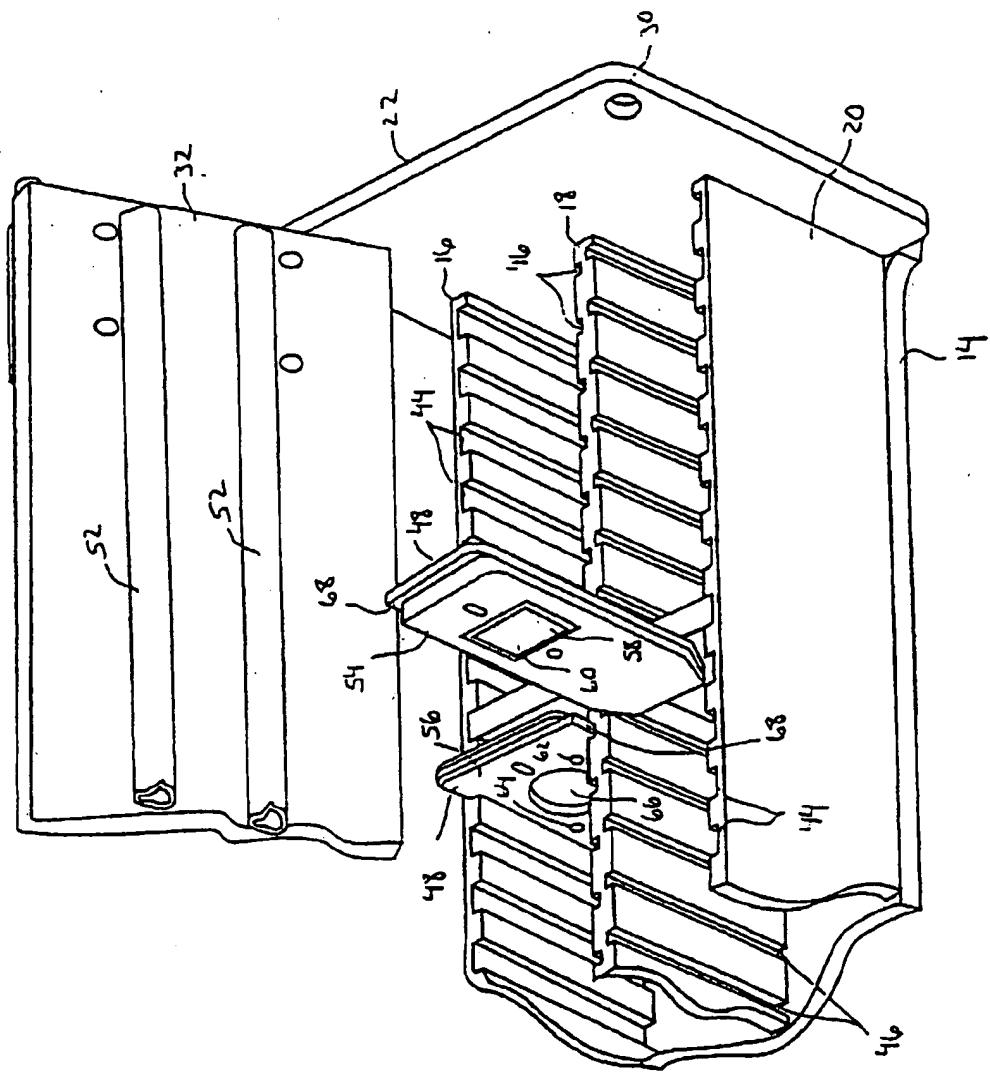
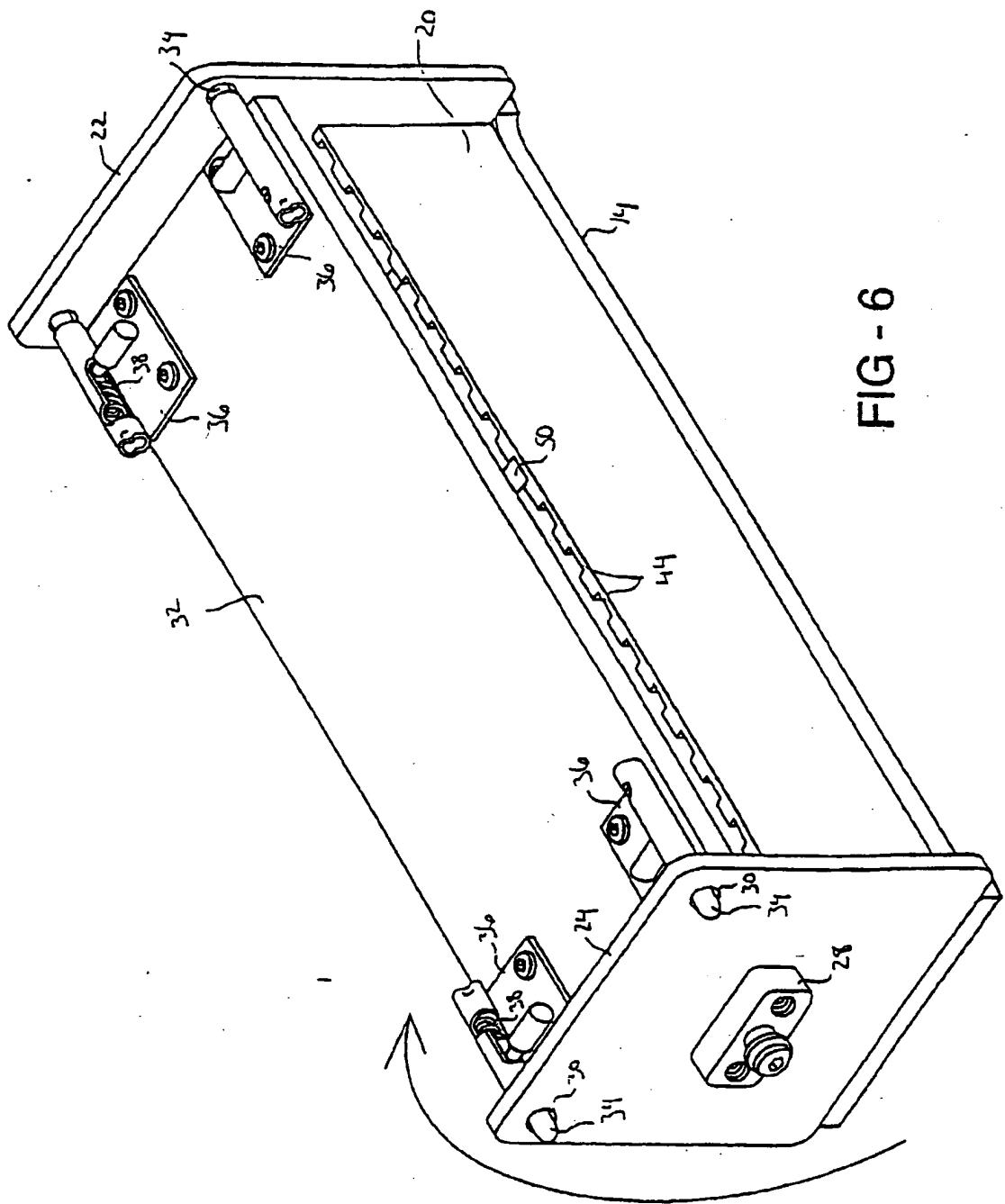


FIG - 4



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FIG





European Patent
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EUROPEAN SEARCH REPORT

Application Number
EP 99 30 0721

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	US 5 162 237 A (LOWRY J- MESSENGER ET AL.) 10 November 1992 * abstract * * column 5, line 51 - column 6, line 39 * * column 7, line 57 - column 8, line 21 * * column 17, line 66 - column 18, line 13 * * column 19, line 43 - column 20, line 5 * * figures *	1	B01J19/28 B01F9/00 C12M1/10
A	---	2,9-13	
A	US 5 665 309 A (HENRI CHAMPSEIX ET AL) 9 September 1997 * the whole document *	1-8, 11-15	
P,A	DE 297 19 099 U (KONRAD THIELE) 12 March 1998 * the whole document *	1	
A	WO 97 21834 A (MJ RESEARCH, INC.) 19 June 1997 * abstract; figures *	1	
A	GB 2 229 814 A (TECHNE (CAMBRIDGE) LIMITED) 3 October 1990 * abstract; figure 2 *	1	B01J B01F C12M G01N A47B B01L
D,A	WO 95 33846 A (AFFYMAX TECHNOLOGIES N.V.) 14 December 1995 -----		
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
THE HAGUE	12 May 1999	Stevnsborg, N	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
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ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

EP 99 30 0721

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

12-05-1999.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5162237	A	10-11-1992	US	4990075 A	05-02-1991
			AU	3256489 A	12-10-1989
			CA	1330197 A	14-06-1994
			EP	0339277 A	02-11-1989
			JP	2022560 A	25-01-1990
			JP	2711354 B	10-02-1998
			US	5084397 A	28-01-1992
			AU	617947 B	05-12-1991
			AU	5779490 A	17-01-1991
			CA	2018323 A	11-01-1991
			EP	0407827 A	16-01-1991
			FI	98862 B	15-05-1997
			JP	3046566 A	27-02-1991
US 5665309	A	09-09-1997	FR	2730315 A	09-08-1996
			BR	9600549 A	30-12-1997
			EP	0726453 A	14-08-1996
			HU	9600204 A	28-07-1997
			JP	8248036 A	27-09-1996
DE 29719099	U	12-03-1998	NONE		
WO 9721834	A	19-06-1997	EP	0868531 A	07-10-1998
GB 2229814	A	03-10-1990	NONE		
WO 9533846	A	14-12-1995	AU	2943695 A	04-01-1996
			EP	0695941 A	07-02-1996
			EP	0764214 A	26-03-1997
			JP	8166387 A	25-06-1996
			JP	10505410 T	26-05-1998